

Antibacterial activity of extracts of leaves of *Nerium indicum* in combination with antibiotics

Piyush Vyas*, Arvind Suthar and Deepkumar Joshi

Chemistry Dept., Sheth M. N. Science College, Patan-384265

E-mail address: vyaspiyushj@yahoo.com

Keywords: Antibacterial activity; *Nerium indicum*; antibiotics

ABSTRACT. Methanol and ethanol extract showed antibacterial activity in range of 0-11 at 10 mg/mL against all tested bacterial species. Methanol extract with amoxicillin exhibited effective inhibition *E. coli*. This combination showed antagonistic effect against *B. subtilis* and *S. aureus*. Interaction of ethanol extract with amoxicillin also showed effective inhibition against *E. coli* and decreased activity against *B. subtilis* and *S. aureus*.

Methanol extract did not show inhibition against *P. aeruginosa* but it enhanced activity of ciprofloxacin on combination. Ethanol extract also exhibited effective inhibition against *P. aeruginosa* on combination with ciprofloxacin. But antagonist effect was observed on interaction of both extract with ciprofloxacin against other bacterial species.

Interaction of methanol and ceftazidime showed mostly decreased or indifferent effect against all bacterial species. Ethanol extract with ceftazidime resulted in higher inhibition against *E. coli*.

Combination of methanol extract with erythromycin showed higher inhibition against *E. coli* and also very effective inhibition against *S. aureus*. This combination resulted in decreased activity against *B. subtilis*. Ethanol extract with erythromycin also showed synergistic effect against *S. aureus* and effective inhibition against *E. coli*. These results indicate that both methanol and ethanol extract has potential to enhance the combination effect with erythromycin against *S. aureus* and *E. coli*.

1. INTRODUCTION

Plants are used for treatment of number of diseases for thousands of years. Plants contain number of active compounds which are responsible for various biological activities and play an important role in conventional as well as western medicine ¹. It is also reported that phytochemicals, which are known as secondary metabolite from plants act as synergists or potentiators of other antibacterial agents ². Antibiotics are very effective for treatment of number of infectious diseases. The emergence and spread of multidrug resistance pathogens have been increasing due to overuse of antibiotics ^{3, 4}. Development of resistance pathogens is a big problem for human health. It is required to find out some alternatives to overcome the problems of multidrug resistance. One approach is use of combination of plant extracts with antibiotics. Many studies indicated that efficacy of antimicrobial agents can be improved by combining them with plant extracts ⁵. Interaction of plant extract with antibiotics leads to new way to treat infectious diseases ⁶.

This study has been done to evaluate combined effect of extracts of leaves of *Nerium indicum* with different antibiotics. *Nerium indicum* belongs to apocynaceae family and it is a large evergreen shrub with milky juice ⁷. Leaves have been applied externally in the treatment of scabies and to reduce swellings. The leaves and the flowers are cardi tonic, diaphoretic, diuretic, emetic, expectorant and sternutatory ⁸. The root powder is an external remedy for hemorrhoids and ulcers around genitals. Leaves and bark is treated as insecticide, rat poison and parasitic ^{9, 10}.

It is previously reported that the alcoholic extract of leaves of *Nerium indicum* was effectively inhibited the growth rate of *Staphylococcus aureus*, *Candida albicans*, *Aspergillus niger*, *Mucor*, *Rhizopus* and *Penicillium species* even at lower concentrations ¹¹. Its leaves also exhibited antioxidant ¹², analgesic ¹³ and antiviral activity ¹⁴.

The leaves of *N. indicum* contain neriin and oleandrin; both are cardiac glycosides with properties similar to digitalin ¹⁵. Leaves also contain ursolic acid that is similar to rutin ¹⁶.

2. MATERIALS AND METHODS

Plant material

Leaves of *Nerium indicum* was collected from local area and identified by expert. Leaves were washed with tap water followed by distilled water to remove dust particles and then dried at room temperature for few days. Properly dried leaves were grinded to form powder and stored it in airtight bottle till use.

Extraction

Powder of leaves was extracted with methanol and ethanol using soxhlet apparatus. The filtrate was evaporated to dryness. Dry extracts was stored properly till use.

Microorganisms

The following four bacterial strains were used in the study - 1. *Bacillus subtilis* (MTCC 441) 2. *Escherichia coli* (MTCC 1687) 3. *Pseudomonas aeruginosa* (MTCC 1688) 4. *Staphylococcus aureus* (MTCC 737)

Antibiotics

Amoxicillin, Cefazidime, Ciprofloxacin and Erythromycin were purchased from *Hi-Media* Laboratories.

Determination of antibacterial activity

In vitro antibacterial activity of the crude extracts was studied by the agar well diffusion method. After getting the turbidity equal to 0.5 McFarland standards, inoculums were aseptically introduced on to the surface of sterile agar plates and sterilized cotton swabs were used for even distribution of the inoculums. Wells were prepared in the agar plates using a sterile cork borer of 8.0 mm diameter. The plant extract and antibiotic drug were dissolved in DMSO to get desired concentration. The wells were filled with plant extract (100µl) and antibiotic drug (100µl). The plates are incubated at 37 °C for 48 hours and then zone of inhibition was measured. In case of combination of plant extract and antibiotic, equal volume (50µl) of each was added in the well and zone of inhibition was measured. The experiment was replicated two times. (Zone of Inhibition = ± 1 mm)

3. RESULTS

Table 1: Antibacterial activity of antibiotics

Bacteria	Zone of Inhibition (mm)			
	Amx	Cipro	Cefta	Eryth
<i>B. subtilis</i>	30	27	5	23
<i>E. coli</i>	6	28	16	8
<i>P. aeruginosa</i>	0	28	8	2
<i>S. aureus</i>	30	27	3	18

(Amox-Amoxicillin; Cipro-Ciprofloxacin; Cefta- Cefazidime; Eryth- Erythromycin. Conc. of antibiotics-50 µg/mL)

Table 2: Combined antibacterial activity of methanol extract plant extract and antibiotics

Bacteria	Zone of Inhibition (mm)				
	ME	M.E + Amx	M.E + Cipro	M.E + Cefta	M.E + Eryth
<i>B. subtilis</i>	10	21	23	7	19
<i>E. coli</i>	10	17	25	12	15
<i>P. aeruginosa</i>	0	0	35	7	0
<i>S. aureus</i>	9	22	26	4	26

(ME-Methanol extract; Conc. of methanol extract- 10 mg/mL)

Table 3: Combined antibacterial activity of ethanol extract plant extract and antibiotics

Bacteria	Zone of Inhibition (mm)				
	EE	E.E + Amx	E.E + Cipro	E.E + Cefta	E.E + Eryth
<i>B. subtilis</i>	9	24	23	7	18
<i>E. coli</i>	10	17	25	23	16
<i>P. aeruginosa</i>	3	0	35	7	0
<i>S. aureus</i>	11	23	24	7	24

(EE-Ethanol extract; Conc. of ethanol extract- 10 mg/mL)

4. DISCUSSION AND CONCLUSION

Amoxicillin is semi-synthetic drug and belongs to class of antibiotics called the Penicillin (β -lactam antibiotic). It is effective against various infections which are caused by wide range of Gram-positive and Gram-negative bacteria in both human and animals¹⁷. Ciprofloxacin is a second generation fluoroquinolone antibiotic with a broad spectrum of antibacterial activity¹⁸. It is effective against a wide variety of gram-negative and gram-positive organisms¹⁹. Erythromycin is a group of drugs called macrolide antibiotic and useful for the treatment of a number of bacterial infections²⁰.

Numbers of antimicrobial compounds are obtained from plants containing secondary metabolites are useful as alternative strategies for treatment of infectious diseases²¹. Plant extracts acts as potential sources of antimicrobial and resistance modifying agents. Studies on synergistic effects of plant extracts with antibiotics have been carried out by numbers of researchers. Mechanism of joint action of plant extracts with antibiotics is still not completely understood²². Ability of plant extracts to potentiate the effect of antibiotics could be new approach to fight against problem of bacterial resistance²³.

The aim of this study was to assess the combined activity of antibiotics and extracts of leaves of *Nerium indicum*.

Methanol and ethanol extract showed antibacterial activity in range of 0-11 at 10 mg/mL at against all tested bacterial species. Methanol extract with amoxicillin exhibited effective inhibition *E. coli*. This combination showed antagonistic effect against *B. subtilis* and *S. aureus*. Interaction of ethanol extract with amoxicillin also showed effective inhibition against *E. coli* and decreased activity against *B. subtilis* and *S. aureus*.

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This study indicates that few combinations of plant extract with antibiotics resulted in positive interaction against some tested bacterial species. Our results revealed the potential of plant extract to enhance combination effect with antibiotics. This new concept of combination therapy may be effective to treat various infectious diseases. It is required to screen out more combination with different antibiotics against numbers of resistant bacteria. This is only *in vitro* study and it is required to carry *in vivo* study. Such combination must be followed by toxicity test and *in vivo* tests to determine its therapeutic application.

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